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Expressions of Gamma-interferon and Interleukin-4 Messenger RNA in Splenic L3T4$^+$ T Cells from *Babesia microti*- and *B. rodhaini*-Infected Mice

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To elucidate the type of L3T4$^+$ T cell developing at the initial phase of infection with *Babesia microti* and *B. rodhaini*, expressions of gamma-interferon (IFN-γ) and interleukin-4 (IL-4) messenger RNA (mRNA) in splenic L3T4$^+$ T cell from *B. microti*- and *B. rodhaini*-infected mice were examined. The strong expression of IFN-γ mRNA was observed in splenic L3T4$^+$ T cell from *B. microti*-infected mice at days 3, 6 and 9 after inoculation, whereas in *B. rodhaini*-infected mice the strong expression of IL-4 mRNA was observed at days 3 and 6 after inoculation. This indicated that the subset of splenic L3T4$^+$ T cells developing at the initial phase of infection was helper T cell type-1 cell, which enhanced cell-mediated immune response in *B. microti*, and helper T cell type-2 cell, which induced humoral one in *B. rodhaini*. Therefore, it was suggested that the different outcome of the infection with *B. microti* and *B. rodhaini* in mice, non-lethal and lethal, respectively, resulted from the development of different subset of splenic L3T4$^+$ T cell at the initial phase of infection.

Intraerythrocytic protozoa, *B. microti* and *B. rodhaini*, cause different course of infection in mice (Cox and Young 1969; Inchley et al. 1987). Briefly, *B. microti* infection is chronic and non-lethal, whereas *B. rodhaini* infection is acute and lethal. Our previous study demonstrated that L3T4$^+$ T cells enhanced cell-mediated immune response at the initial phase of infection in *B. microti*- but not in

B. rodhaini-infected mice (Shimada et al. 1996). Therefore, the difference of the roles of $L3T4^+$ T cells in the protective cell-mediated immune response at the initial phase of infection might be related to the difference in the course of infection with these two *Babesia* .

It has been well-established that $L3T4^+$ T cell can be divided into two subsets, helper T cell type-1 (Th1 cell) and type-2 (Th2 cell), on the basis of their cytokine production and regulation of immune responses (Powrie and Coffman 1993; Romagnani 1992; Schmitz et al. 1993). Briefly, the former cell produced IFN-γ and interleukin (IL)-2, and regulated cell-mediated immune response, while the latter cell produced IL-4, IL-5, and IL-6, and regulated humoral one. In addition, the contribution of Th1 and Th2 cells to the resolution or progression of infection have been documented in several parasitic infections (Chan 1993; Chensue et al. 1993; Cox and Liew 1992; King and Nutman 1991; Locksley and Scott 1991). Therefore, to clarify the type of $L3T4^+$ T cell subsets developing at the initial phase of infection with *B. microti* and *B. rodhaini*, the expressions of IFN-γ and IL-4 mRNA in splenic $L3T4^+$ T cells from *B. microti*- and *B. rodhaini*-infected mice were examined by reverse transcriptase-polymerase chain reaction (RT-PCR) technique.

Male BALB/c mice aged 8 weeks were inoculated by peritoneal injection with $1x10^4$ per head of *B. microti*- or *B. rodhaini*-parasitized erythrocytes. Splenic cells were prepared at an interval of 3 days until day 12 after inoculation as described previously (Shimada et al. 1992). Then, the $L3T4^+$ T cells were collected by panning method (Wysocki and Sato 1978). Briefly, the bottom of a polystyrene Petri dish (bacteriological grade) was covered with 5 ml of anti-L3T4 mAb solution (GK1.5, IgG2b: kindly provided from Dr. R. Lelchuk, Welcome Research Laboratories, Beckenham, Kent, U.K., reconstituted into 50 mg/ml with 0.01 M PBS, pH 7.4). The dish was stood overnight at room temperature (RT). After discarding the supernatant, the bottom of the dish was covered with 0.2% bovine serum albumin in PBS (0.2% BSA-PBS) for 30 min at RT. Then, 4 ml of $5x10^6$ cells/ml splenic cell suspension in 0.2% BSA-PBS were placed on the dish and incubated for 60 min at 4 °C. After incubation, the dish was washed 3 times with PBS to remove non-adherent cells. Adherent cells were collected by scraping the bottom of the dish with a rubber policeman. The percent of $L3T4^+$ T cells in the adherent cells was determined by flow cytometric analysis as described previously (Shimada et al. 1992) and the adherent cells including more than 90% of $L3T4^+$ T cells were used in RT-PCR assay.

The RNA was obtained from collected cells as follows. The 10^7 cells were solubilized with solution-D (containing 4 M guanidinium thiocyanate, 25 mM sodium citrate at pH 7.0, 0.5% sarkosyl, and 0.1 M 2-mercaptoethanol) and then

RNA was isolated by phenol/ chloroform/ isoamyl extraction and precipitated by isopropyl alcohol. The precipitated RNA was washed with ethyl alcohol, solubilized with solution-D, and reprecipitated by isopropyl alcohol. After washing with ethyl alcohol, RNA was resuspended in diethylpyrocarbonate-treated water. RNA concentration was determined by absorbance at 260 nm. The strand synthesis of cDNA of the extracted total RNA was performed by incubating total RNA with 0.125 mM oligo $d(T)_{16}$, 5 U of cloned moloney murine leukemia virus reverse transcriptase (United states Biochemical Corp., Cleveland, USA), 4 mM dNTP, 5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1 mM dithiothreitol, and 1 U of RNase inhibitor (Toyobo Inc., Tokyo, Japan) for 20 min at 42 °C. The cDNA product was then amplified with 2.5 U of Taq DNA polymerase (Perkin Elmer Cetus Crop., Norwalk, USA), 5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, and 0.5 pmol/ml of each up/downstream primer using a Program Temp Control System PC-700 (Astec Inc., Tokyo, Japan). The following primer pairs kindly provided from Drs. Yoshitsugu MATSUMOTO, Department of Applied Immunology, University of Tokyo, and Takashi YOKOTA and Ken-ichi ARAI, Department of Molecular and Developmental Biology, Institute of Medical Science, University of Tokyo, were used: upstream primer 5'-CGCTACACACTG CATCT TGG-3' and downstream primer 5'-GGCTGGATTCCGGCAACA-3' for IFN-γ, and upstream primer 5'-GTCACAGTTTTCAGCTGTATAGGG-3' and downstream primer 5'-AACACCACAGAGAGTGAGCTCGTCT-3' for IL-4. The program consisted of 1 cycle of 95 °C for 2 min, followed by 30 cycles of 95 °C for 1.5 min, 30 cycles of 60 °C for 1.5 min, and 1 cycle of 60 °C for 1 min. The PCR products were electrophoresed on 2% agarose gel and visualized by ethidium bromide staining.

The expression of both IFN-γ and IL-4 mRNA in splenic $L3T4^+$ T cells from *B. microti*-infected mice were observed at day 3 after inoculation, whereas only IFN-γ mRNA was detected at days 6 and 9 after inoculation (Fig. 1). Since Th1 and Th2 cell were reported to differentiate from Th0 cell that possesses the cytokine profiles of both Th1 and Th2 cell (Mosmann and Moore 1991), the subset of splenic $L3T4^+$ T cell at day 3 after inoculation was considered to be Th0 cell. The expression of only IFN-γ mRNA at day 6 and 9 after inoculation indicated the development of Th1 cell in the spleen at the initial phase of *B. microti* infection. Some investigators suggested that the subset of splenic $CD4^+$ ($L3T4^+$) T cell was switched from Th1 to Th2 cell during the course of *Plasmodium chabaudi* AS infection (Stevenson and Tam 1993; Taylor-Robinson and Phillips 1992). In the present study, both IFN-γ and IL-4 mRNAs were expressed again at day 12 after inoculation. Therefore, the subset of splenic $L3T4^+$ T cell might be switched from Th1 to Th2 cell at day 12 after inoculation

in *B. microti* infection.

On the other hand, L3T4$^+$ T cells from *B. rodhaini*-infected mice expressed IL-4 mRNA and also IFN-γ but with a very weak intensity at day 3 and 6 after inoculation. This indicated that Th2 cell was predominant in the spleen at the initial phase of *B. rodhaini* infection, as supported by the observation that L3T4$^+$ T cell from mice susceptible to *Leishmania major* infection produced large amount of IL-4 within 3 days after inoculation (Locksley and Scott 1991; Scott 1991; Scott et al. 1989). Additionally, the expressions of only IFN-γ mRNA was detected at days 9 and 12 after inoculation, indicating the change of T cell subset from Th2 to Th1 cell.

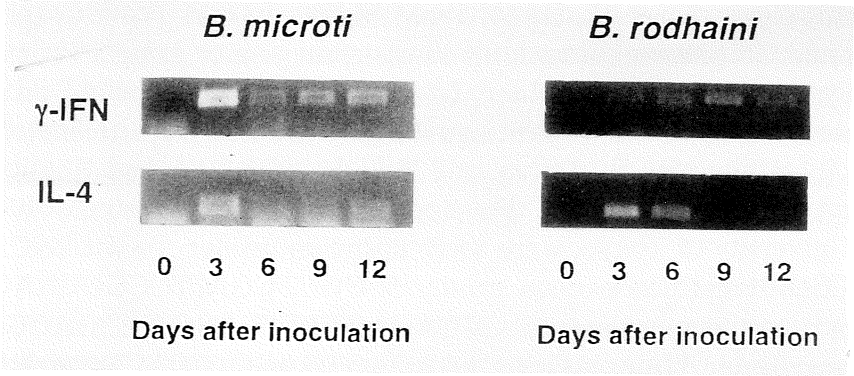

Figure 1. Expressions of IFN-γ and IL-4 mRNA in splenic L3T4 positive T cells after *Babesia microti* and *B. rodhaini* inoculation.

The present data demonstrated that the subset of splenic L3T4$^+$ T cell developing at the initial phase of infection was predominantly Th1 cell in *B. microti*-infected mice, and Th2 cell in *B. rodhaini*-infected mice, respectively. In *Leishmania major* infection, Th1 cell was activated in resistant mice at the initial phase of infection, whereas Th2 cell developed in susceptible mice (Heinzel et al. 1989; Locksley and Scott 1991; Scott 1991). Furthermore, Stevenson et al. suggested that induction of a strong Th2 cell response at the early prime of infection lead to a lethal course of malaria. Therefore, it was suggested that the different course of infection between non-lethal *B. microti* and lethal *B. rodhaini* infection in mice resulted from the development of different subset of splenic L3T4$^+$ T cell at the initial phase of infection.

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